Genome editing weds CRISPR: what is in it for phytoremediation?

CRISPR has transformed the genome editing process. CRISPR could be used to modify and regulate phenomenon of our interest in organisms from all three domains of life. Applications of CRISPR mediated precision genome engineering are immense and we are optimistic that it marks the dawn of a remarkable era of genome reprogrammed achievements. In this perspective piece, we postulate how the scientific breakthrough of CRISPR-Cas9 facilitated genome engineering could help in achieving sustainable environmental cleanup via phytoremediation.

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Genome editing weds CRISPR: what is in it for phytoremediation?

Zarrin Basharat*, Azra Yasmin

Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi 46000, Pakistan.

*Corresponding author:

Zarrin Basharat

Punjab, Rawalpindi 46000, Pakistan.

E-mail address: zarrin.iiui@gmail.com

Abstract

In this perspective piece, we postulate how the scientific breakthrough of CRISPR-Cas9 facilitated genome engineering could help in achieving sustainable environmental cleanup via phytoremediation.

Keywords: Genomics, CRISPR, phytoremediation.

Advent of the prokaryotic immune system centred clustered regularly interspaced short palindromic repeats i.e. CRISPR technology has breathed a new life into the genome editing endeavours (Pennisi, 2013). CRISPR-Cas has been harnessed for generating knock-outs, targeting transcriptional regulation or make substitutions in the genome (Sander & Joung, 2014). This system is centred on a guide RNA (gRNA) facilitated direction of the bacterial Cas9 nuclease to a target site (Hsu *et al.,* 2013). Substantial progress is being made increasingly in this realm as tools and methods of CRISPR-Cas9 usage in genome editing continue to expand due to considerable advantage over competing techniques. However, usage of this method for genome engineering aimed at phytoremediation is underrepresented, despite being need of the hour. We have highlighted the importance of this effective tool for precision genome editing of plants important for remediation of toxic contaminants (comprising organic, inorganic substances as well as radionuclides).

Phytoremediation is a green, solar energy driven, low cost technology that exploits plants to mitigate the impact of harmful pollutants (Khandare & Govindwar, 2015). It also helps refurbish natural habitats and heal the hideous scars of the landscape. Important properties of phytoremediators that favour swift remediation include faster growth rate, high biomass, hardiness and bioconcentration (Cherian & Oliveira, 2005). Taking these properties into account, several plant species have been identified as attractive candidates for the purpose of phytoremediation and further enhanced by scientists via transgenics (Reviewed by Cherian & Oliveira, 2005). Plants exhibiting enhanced ability to accumulate pollutants (50-500 times more than average plants) i.e. hyperaccumulators are particularly appealing for environmental cleanup (Cherian & Oliveira, 2005; Lasat, 2000).

Genome editing of plants for phytoremediation using CRISPR-Cas systems is an unexplored yet a promising venture to increase remedial capacity of plants. Genomes of model hyperaccumulators *Noccaea caerulescens* (formerly known as *Thlaspi caerulescens*),

Arabidopsis halleri, *Pteris vittata*, *Brassica juncea*, *Populus trichocarpa* and several other phytoremediators have been sequenced. Few energy crops have also been sequenced (Estrela & Cate, 2016) and editing their genomes for increased tolerance to pollutants could deliver multiple benefits. Manipulation of genomic sequences of these plants may facilitate the identification and characterization of key genetic determinants in the investigation of phytoremediation processes like phytoextraction, phytoaccumulation, phytovolatilization etc. Sequence data information of these plants can be utilized to establish CRISPR-Cas9 systems for phytoremediation by targeted engineering of mechanisms involved in the accumulation, complexation, volatilization, and degradation of pollutants. CRISPR-Cas9 systems for genome modification of efficient hyperaccumulator *Populus* specie already exists (Fan *et al.*, 2015) and can be extended for other plants of interest.

CRISPR could be used to transfer a desired set of instructions in the plant genome in a candid mode as it is a programmable, next-generation method for high throughput genetic manipulation as compared to the low throughput zinc finger nucleases and TAL effectors (Jinek *et al.,* 2012*;* Mali *et al.,* 2013). Sequence availability of plant genomes aided by software tools, bioinformatics based approaches and availability of codon-optimized versions of Cas9 for monocots as well as dicots has opened new avenues for using CRISPR-Cas9 genome editing in a wide variety of plants (Lowder *et al.*, 2015). Areas of focus for phytoremediation may include enhanced expression of enzymes (such as metallothioenins, phytochelatin synthases, metal reductases etc), pathways necessary for pollutant transport such as vacuolar compartmentation of heavy metals to avoid disrupting other cellular functions and production and transport of undesired metabolites to the rhizosphere. Simultaneous elimination of a class of pollutants or several type of pollutants could also be probed efficiently using CRISPR technology. The outline of the several strategies that could be tested for enhancing phytoremediation include direct transfection of Cas9 along with

gRNAs into the plant protoplasts, plant regeneration from single-cells, T-DNA-delivered gRNA–Cas9, modular cloning systems like Golden-Braid or even via cloning free strategy. gRNA-Cas9 mediated genome editing has been carried out earlier in selected plants but with low efficiency, whereas it has proved absolutely non feasible for several plant species (Belhaj *et al.,* 2015; Vazquez-Vilar *et al.,* 2015). T-DNA-delivered gRNA-Cas9 (in *Agrobacterium* mediated T-DNA transformation) has also been tested but due to transitory action of T-DNA in callus induction, activity has been observed in somatic tissues via genome integration. To make the most of this strategy, it might be imperative to amalgamate diverse gRNAs with Cas9 in a single T-DNA, as all-in-one plasmid approach would definitely improve editing (Bortesi & Fischer, 2015; Mikami, Toki & Endo, 2015). Cloning systems like Golden-Braid ease the association of pre-made DNA elements into multigene constructs (Vazquez-Vilar *et al.,* 2015; Liu & Stewart, 2015). Multiplexed editing regulatory assays employ a cloning-free strategy to ensure incorporation of a single gRNA in the cells but impact throughput.

CRISPR-Cas9 system has also been successfully employed in the past for genome editing in several plant species of interest (food and energy crops) (Estrela & Cate, 2016; Song *et al.,* 2016). Owing to complexities in plant genomes e.g high ploidy, traditional breeding experiments take long turnaround times for each experiment as well as low frequency of homologous recombination makes site-specific mutagenesis difficult (Estrela & Cate, 2016). Growing need for increasing plant biomass, growth rate, disease resistance, hyperaccumulation and coupling these traits to harvesting other products of interest could be wondrous as gRNA-Cas9 facilitates targeting multiple sequences and hence, traits simultaneously.

Although CRISPR/Cas9 has shown promise for genome engineering, results depend on choice of the target site, Cas9 action, design of gRNA, delivery systems as well as off-target outcomes may impede progress (Peng, Lin & Li, 2016). However, we are hopeful that things

will improve with time as our understanding of the system increases. Overall, CRISPR aided genome engineering heralds' great potential for exploiting plant genomes for enhanced phytoremediation. Modifying genes of interest, their expression, whole pathway and pollutant homeostasis networks that support hyperaccumulation or tolerance can be revolutionary for cleaning environment via plants. We are hopeful that this technique could deliver robust traits, leverage efficiency of desired characteristics such as increased plant biomass along with other traits in a single go and appears to take phytoremediation to its zenith.

Competing interests

The authors declare that no competing interests exist.

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